

Department of Environmental and Ecological Sciences  
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# **Remediation of hydrocarbon contaminants in cold environments – electrokinetically enhanced bioremediation and biodegradable oil sorbents**

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ACADEMIC DISSERTATION

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## Abstract

Owing to the vast amounts of oil in the world, oil spills are common on land as well as at sea. In addition to oil products, other industrially used hydrocarbons, such as creosote, also contaminate soils. Most hydrocarbons are biodegradable. Hence, bioremediation is an attractive alternative for cleaning up hydrocarbon spills. In cold climate areas, however, biodegradation is often a slow process. The aim of this thesis was to develop efficient, cost-effective, and ecologically sound techniques for cold climate areas for the treatment of both oil spills on water or solid surfaces and for subsurface contamination.

For subsurface hydrocarbon spills, the approach was to use electrokinetics in order to deliver nutrients and possibly microorganisms to contaminated soils, and in order to heat the soil to increase contaminant bioavailability and microbial activity. Electroosmosis proved to be an effective method to disperse bacteria and nutrients in medium- or fine-grained soils, where uncharged particles migrated with water through the soil towards the cathode. Creosote degradation proceeded faster with electroosmotically added nutrients than in controls without additions. However, inoculation with enriched creosote-degraders was not necessary because the indigenous microbes of the soil were well adapted to the creosote contaminants.

For small-scale oil spills, the usual remediation method involves absorption with oil sorbents. Most sorbents in use today are synthetic and incineration is the only method for their disposal. A biodegradable sorbent, however, could be processed, for instance, in compost-like systems. Cotton grass is a common plant in peat bogs and its water-repellent fibre is a by-product of peat excavation. Cotton grass proved to be an excellent oil sorbent especially for spills on the surface of water: It absorbed up to three times as much oil as a commercial, synthetic oil sorbent. Cotton grass performed extremely well also in conditions simulating winter in the Baltic Sea. In addition, the effect of drying microbial suspension on an oil sorbent to enhance oil degradation of the oily sorbents was investigated. Microbial treatment of oil sorbents could be beneficial in mineral soils with low initial microbial density if fast degradation is of importance. Otherwise, it may not be worthwhile.

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## List of original publications

This thesis is based on the following publications:

- I. Suni, S. and Romantschuk, M., 2004. Mobilisation of bacteria in soils by electro-osmosis. *Microbiol. Ecol.*, 49, 51-57.
- II. Suni, S., Malinen, E., Kosonen, J., Silvennoinen, H. and Romantshuck, M., 2007. Electrokinetically enhanced bioremediation of creosote-contaminated soil: laboratory and field studies. *J. Environ. Sci. Health, Part A* A42 (7), in press.
- III. Suni, S., Kosunen, A.-L., Hautala, M., Pasila, A. and Romantschuk, M., 2004. Use of a by-product of peat excavation, cotton grass fibre, as a sorbent for oil spills. *Mar. Pollut. Bull.*, 49, 916-921.
- IV. Suni, S., Kosunen, A.-L. and Romantschuk, M., 2006. Microbially treated peat-cellulose fabric as a biodegradable oil-collection cloth. *J. Environ. Sci. Health, Part A* A41 (6), 999-1007.

The publications are referred to in the text by their roman numerals.

# 1. Introduction

The world depends on oil. Vast amounts are used, transported, processed, and stored all around the world. In 2003, the total world consumption of petroleum was over 13.1 billion litres per day (EIA, 2006). With such a high consumption, oil spills are inevitable. Even virtually inhabited areas, such as Antarctica, cannot escape oil spillages (e.g. Kennicutt, 1990). However, not all hydrocarbon contamination is anthropogenic: The contribution of natural sources to oil pollution in seas is 47% (NAS, 2003). Nevertheless, reducing oil and other hydrocarbon contamination from anthropogenic sources is vital for the protection of the environment.

In Finland, the number of suspected contaminated sites is approximately 20 000, of which one third is contaminated with petroleum products and approximately 6% with creosote (Finnish Environment Institute, 2006; Puolanne et al., 1994). Remediation practices of contaminated soils lag behind those of most other countries in Europe. Only after the 1994 assessment of the extent of soil contamination in Finland (Puolanne et al., 1994), did the number of decisions to remediate contaminated sites increase. Today, about 400 contaminated sites are remediated every year. At the beginning of the last decade, the number was approximately ten sites a year (Finland Environment Institute, 2006). At some of these sites, the contamination has occurred recently but at others several years or even decades ago. Even at old sites, the risk of a serious environmental hazard may become acute if recalcitrant compounds in the soil leach into the groundwater. The most common chemicals contaminating soils, groundwater, and sediments in Finland are petroleum products, heavy metals, and chlorinated hydrocarbons (Finnish Environment Institute, 2006; Puolanne et al., 1994). Those mostly responsible for the contamination are service stations, garages, scrap yards, waste treatment plants, sawmills, and wood impregnation plants are mostly (Mroueh et al., 1996; Penttinen, 2001; Puolanne et al., 1994).

The most notable oil spills at sea involve large tankers, such as Prestige or Exxon Valdez, that spilled thousands of tonnes of oil (Albaiges, 2006; Paine et al., 1996). These oil spills can cause severe damage to sea and shoreline organisms (Whitfield, 2003). However, the contribution of tanker catastrophes to the total oil load of the seas is only 4-13%. The majority of the anthropogenic oil contamination originates from illegal discharges of oily wastes, surface run-offs, and deposits from air (NAS, 2003). Finland is partly bordered by the Baltic Sea, through which passes 15% of the world's maritime traffic (HELCOM, 2003). Several important oil ports are situated in the Gulf of Finland and the amount of annually transported oil is more than 100 million tonnes. Up to 70 000 tonnes of oil ends up in the Baltic Sea each year, and the number of illegal discharges could be as high as 10 000. (HELCOM, 2003; Hänninen and Rytönen, 2004). The Baltic Sea is a very challenging environment because of its slow water turnover, variable salt concentration, lack of oxygen, atypical fauna, and cold climate. It is a small and partly closed sea, which enables hazardous substances to persist a long time. It is one of the world's largest brackish water areas and its water is layered according to salt

concentration. This layering inhibits effective mixing of oxygen in the water. The Baltic is a young sea, only about 8 000 years old (Elmgren, 2001). From an evolutionary viewpoint, its fauna has had only a short time to adapt and represents an atypical mixture of fresh and salt-water organisms. Because of short food chains, hazardous substances become easily enriched in the higher organisms. Accumulation of hazardous substances to organisms and bottom sediments is greater in the Baltic than in the oceans because cold climate, ice cover in winter months, and slow turnover of the water inhibit degradation.

Finnish soils have mainly developed during and after the latest Ice age, and the average thickness of the soil is only a few metres. About half of the country is covered by glacial till, moraine. The majority of the till is silty or sandy with a permeability coefficient as low as 10<sup>-6</sup>-10<sup>-9</sup> cm/s (Heikkinen, 2000). The soils in Finland are cold: The mean annual temperature of soils at 50 cm below soil surface ranges from 1.9 °C to 6.4 °C (Yli-Halla and Mokma, 1998). The water reservoirs in Finland are usually small and lie generally 2-5 m below surface. Regarding contamination migration, the most critical soils are coarse gravel and sand soils with high hydraulic permeability and low contaminant sorption. They also constitute the main exploited groundwater aquifers in Finland (Heikkinen, 2000).

Prevention is the key to combating hydrocarbon contamination. However, efficient, reliable, economically and ethically sound methods are also necessary to clean up, i.e. remediate, environments contaminated with oil compounds, when oil spills do occur.

## **1.1. Behaviour and effects of oil and creosote**

The behaviour of hydrocarbons in soil depends on the characteristics of the substance, such as density, solubility, volatility, and biodegradability, as well as on soil type and on environmental conditions, such as permeability, pH, redox-potential, temperature, and moisture. Hydrocarbons can exist in soil in four phases: adsorbed on soil particles and colloids (especially on clay particles and organic matter), as an immiscible liquid in the soil pore spaces (non-aqueous phase liquid, NAPL), as vapour in the pore space, or dissolved in soil pore water or groundwater. They may also exist as a floating plume above the water table (light non-aqueous phase liquid, LNAPL) or as a sinking plume (dense non-aqueous phase liquid, DNAPL) at the bottom. The partitioning of contaminants may vary as conditions on the site change (Jeltsch 1990; Suthersan 1999).

At sea, oil quickly forms a very thin layer on the surface of water, spreads to a large area, and begins to weather. Various physical, chemical, and biological processes change its composition. The most important processes include evaporation, mixing, emulsification, degradation, and sinking (Douglas et al., 2002; Nicodem et al., 1997). Only in a few days after an oil spill, 75% of the light oil products, up to 12 carbons, can evaporate leaving the heavier and more persistent compounds behind (Prince, 1997). This residual oil has a considerably higher density than the original oil, even higher than water.

As a result, it sinks. The most important environmental factors affecting the behaviour of oil are wind, waves, and air temperature. Strong wind and waves increase mixing causing oil to disperse into the water column and to form an emulsion (Dutta and Harayama, 2000; Nicodem et al., 1997). An additional, major factor affecting the behaviour of oil in the Baltic Sea during winter is ice coverage.

Creosote is a toxic and carcinogenic substance used in wood impregnating plants as a wood preservative (ATSDR, 1996; Kulik et al., 2006). It can contain up to 200 compounds, the approximate composition by mass is 85% polycyclic aromatic hydrocarbons (PAHs), 10% phenolic compounds, and 5% oxygen-, sulphur-, and nitrogen-heterocyclic aromatic compounds (Mueller et al., 1989). Of the total weight of typical creosote, between 20% and 40% can be attributed to the 16 PAHs defined as priority pollutants by the United States Environmental Protection Agency (EPA) (Kulik et al., 2006). Creosote has a strong unpleasant odour and it has been linked to symptoms including skin itch, headache, eye burning, sore throat, nausea, cough, and chest tightness (Dahlgren et al., 2003). After 2003, creosote can be used only industrially in Finland.

Crude oil and oil products contain hundreds or thousands of aliphatic, branched, and aromatic hydrocarbons (Prince, 1993; Wang et al., 1998), most of which are toxic to living organisms (ATRDS, 1995). Fat-soluble components may accumulate in the organs of animals and may be enriched in the food chain, even up to humans (Mackay and Fraser, 2000). In addition to toxic effects, oil products can affect plants and animals physically. A thick layer of oil inhibits the metabolism of plants and suffocates them. Destruction of plants affects the whole food web and decreases the natural habitats of numerous species (HELCOM, 2003). To animals, even a slight staining by oil can be fatal (Wells, 2001). Oil stains can decrease the insulation of a bird's feathers causing the bird to freeze to death. In addition, oil diminishes the ability of birds to fly, dive, and swim, which leads to starvation. Birds also swallow oil when cleaning their feathers, which causes intoxication (EPA, 1999). In the long term, toxic and carcinogenic compounds can cause intoxication, diseases, cell damage, developmental disorders, and reproduction problems. Carcinogenic effects may become evident only generations later (ATRDS, 1995).

## **1.2. Remediation of hydrocarbon-contaminated environments**

Remediation refers to removing, degrading, or transforming contaminants to harmless or less harmful substances. Additionally, it includes methods that reduce mobility and migration of the contaminants, preventing their spreading to uncontaminated areas; toxicity of the contaminants remains unaltered, but the risk they pose to the environment is reduced (US DOD, 1994). Most remediation practices of underground contamination rely on excavating the soil and treating it in separate areas or treatment facilities. These treatments include, for example, thermal treatment and land filling. Incineration is a very effective treatment method, but it is costly and after burning, the soil has lost most of its nutritional value and structure. Land filling does remove the contaminants but only



relocates the problem (Lageman et al., 2005). Furthermore, in Europe, legislation requires reduction of the number of landfills. In 2004, the number of landfills permitted to receive untreated contaminated material in England and Wales was reduced from over 200 to only eleven (EA, 2006). In Finland, the number of landfills has decreased from 232 in 1999 to 80 in 2005 (FEI, 2006). As a result, the cost of dumping contaminated soils into landfills has risen considerably. It is therefore evident that new, innovative methods are needed to treat contaminated soils.

### **1.2.1. Bioremediation of hydrocarbon spills**

Bioremediation refers to cleaning contaminated environments with the aid of microorganisms that transform contaminants to harmless or less harmful compounds. Most hydrocarbons are biodegradable. The unbranched, small chain-length alkanes (C10-C26) are usually considered readily degradable. Low-molecular-weight aromatics, such as benzene, toluene, and xylene are also rapidly biodegraded (Atlas, 1995; Wang et al., 1998). The light aromatics are also among the most toxic petroleum compounds (ATSDR, 1995). The simpler the structure and smaller the compound, the more easily it is biodegraded. Thus, the higher the molecular weight, level of substitutions, or polycyclic aromatic structures, the slower the biodegradation (Atlas, 1995; Wang et al., 1998).

Hydrocarbon-degrading microorganisms are ubiquitous in nature (Atlas, 1981). However, the percentage of hydrocarbon degraders of all the microbial strains in the soil or water is quite low (< 1%) if there is no contamination in the area, but after an oil spill, their level may increase 1 000 –fold (Atlas, 1981; Prince, 2005). Environmental conditions and properties of the contaminated ecosystem heavily affect the degradation of oil hydrocarbons. A given compound can be readily degraded at one site and persist for a long time in another if the conditions differ. Low temperature, for example, decreases the activity of microorganisms (Atlas, 1981; Foght et al., 1998). On the other hand, microbes adapted to low temperatures and able to maintain metabolic activities even in subzero conditions do exist in cold environments. These microorganisms are called psychrophilic or psychrotolerant (Morita, 1975). Among psychrotolerants, many have proven to be hydrocarbon degraders: e.g. alkane degraders (Bej et al., 2000; Ruberto et al., 2005; Whyte et al., 1998), aromatic degraders (Aislabie et al., 1999; Baraniecki et al., 2002; Grishchenkov et al., 2003; Margesin et al., 2005), and chlorophenol degraders (e.g. Järvinen et al., 1994; Männistö et al., 2001; Tirola et al., 2002). Although psychrotolerant bacteria are viable and active in near zero temperatures, the rate of their metabolism is quite low. Even a slight increase in temperature could, therefore, increase their activity and the degradation rate of possible contaminants.

Bioremediation offers three main approaches for treating contaminants, i.e. natural attenuation, biostimulation, and bioaugmentation. In natural attenuation, the naturally occurring, i.e. indigenous, microorganisms degrade the contaminants without any additions and the rate and level of degradation is carefully monitored. However, in cold climate areas, such as Finland, natural attenuation is slow. In wintertime, the microbial

activity virtually ceases (Romantschuk et al., 2000; Schiewer and Niemeyer, 2006). In biostimulation, indigenous microorganisms are stimulated by the addition of nutrients and often of electron acceptors (Atlas, 1995; Foght et al., 1998). Hydrocarbons do not contain substantial amounts of any other essential nutrients for microbial growth besides carbon (Prince, 2005). In case of a spill, hydrocarbons serve as the carbon and energy source but often the lack of other nutrients will soon hinder the growth and metabolism of degrading microorganisms and, thus, active biodegradation of the compounds (Romantschuk et al., 2000). For the last two decades, an increasing interest on anaerobic degradation of hydrocarbons has risen. Studies have revealed that hydrocarbons can be degraded under sulfate-reducing (Coates et al., 1996; Meckenstock et al., 2000; Roychoudury and Merret, 2006; Zhang et al., 2000), nitrate-reducing (Burland and Edwards, 1999; Coates et al., 2001; Rockne et al., 2000), iron-reducing (Anderson et al., 1998; Jahn et al., 2005; Roychoudury and Merret, 2006) and methanogenic (Chang et al., 2006; Salminen et al., 2004) conditions. Still, electron acceptors are required, because even though anaerobic degradation of hydrocarbons does occur, the majority of microorganisms degrade oil via aerobic pathways. However, if the site is, for example, a shoreline with waves constantly crashing to the beach, addition of oxygen is probably unnecessary (Atlas 1995; Romantshuck, 2000). In bioaugmentation, contaminant degradation is enhanced by introducing microorganisms into the sites via inoculation (Atlas, 1995; Foght et al., 1998; Vogel, 1996). This inoculum can be a commercial product designed to degrade effectively the target contaminant of the site. On the other hand, these commercial products are not common and often have viability problems once introduced, because they are not adapted to the specific conditions prevailing at the site and often fail to thrive there (Haines et al., 2005; Prince, 1997; Vogel, 1996). Another possibility is to enrich indigenous microorganisms from the contaminated site with the target contaminant and, thus, producing a mixed population of well-adapted microorganisms with a good degradation capability. Bioaugmentation may be an option if the site lacks microorganisms capable of degrading the target contaminant. For example, if contamination is fresh and the natural microflora has, therefore, not had time to adapt to it, and fast remediation is desirable (Foght et al., 1998; Vogel, 1996).

### **1.2.2. *In situ* –remediation of underground spills**

In *in situ* –remediation, the soil is not excavated but treated as it is. This usually leads to considerable savings because excavation costs and costs of transport to treatment facilities are spared. According to the calculations of the Federal Remediation Technologies Roundtable (FRTR, 2000), the costs of biological *in situ*- treatments were approximately 8-80 €/m<sup>3</sup>, the costs of biological *ex situ* –treatments were about 100-200 €/m<sup>3</sup>, and costs of physical, chemical, or thermal *ex situ* -treatments around 160-800 €/tn. The same cost and environmental savings as in *in situ* -soil remediation can be achieved with *in situ* - groundwater treatments as opposed to conventional pump-and-treat systems. Table 1 provides a general overview of common *in situ* –remediation methods.

**Table 1.** Common *in situ* –remediation methods and their benefits and challenges. Adapted from Penn et al. (2002).

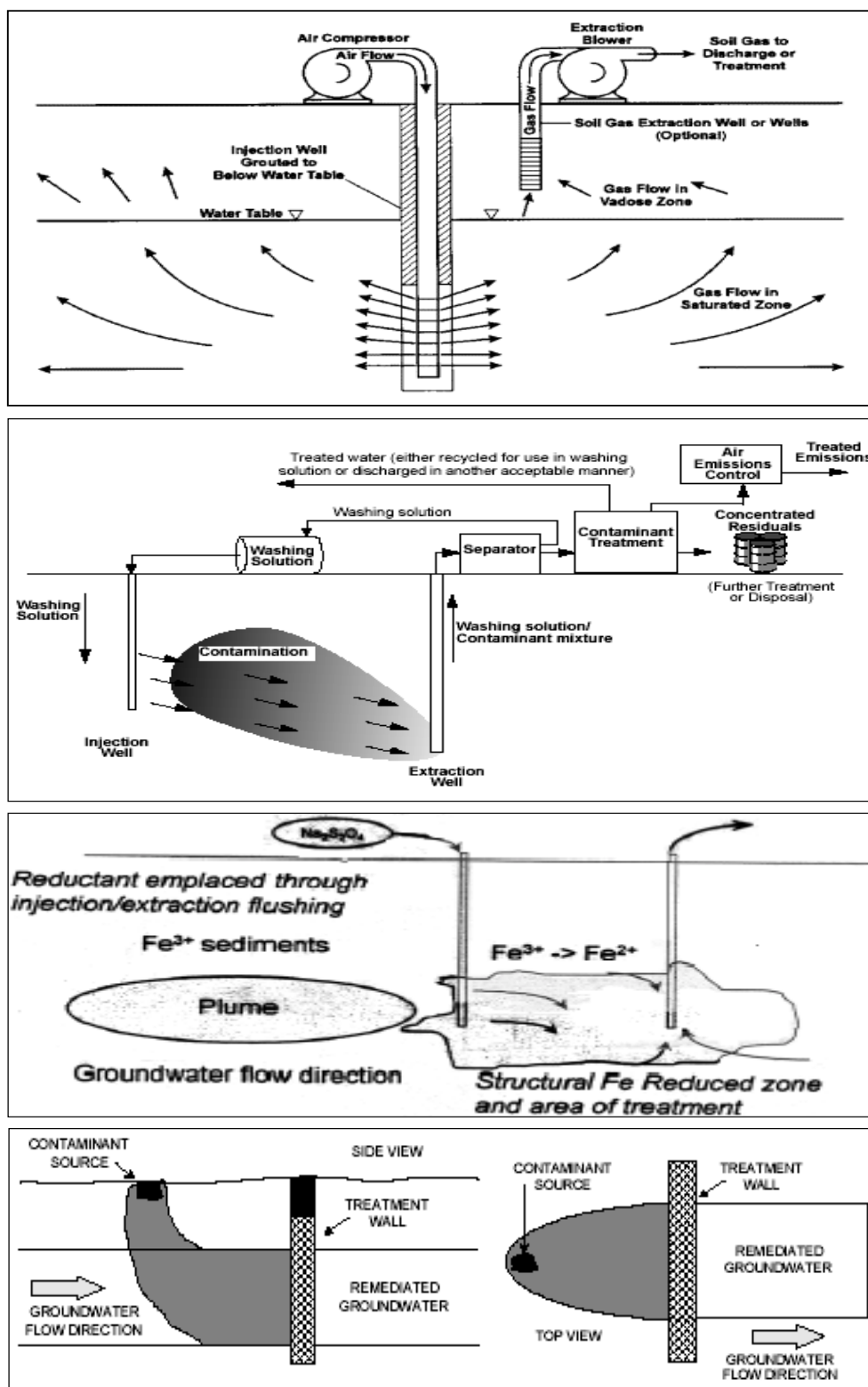
Remediation method	Benefits	Challenges	Examples
Bioremediation (remediation with microorganisms)	<ul style="list-style-type: none"> <li>- Cost-effective</li> <li>- Ecological</li> </ul>	<ul style="list-style-type: none"> <li>- Possibly a slow process</li> <li>- Possible high monitoring costs</li> </ul>	<ul style="list-style-type: none"> <li>- Natural attenuation</li> <li>- Biostimulation</li> <li>- Bioaugmentation</li> </ul>
Phytoremediation (remediation with plants)	<ul style="list-style-type: none"> <li>- Cost-effective</li> <li>- Ecological</li> </ul>	<ul style="list-style-type: none"> <li>- Possibly a slow process</li> <li>- Possible high monitoring costs</li> </ul>	<ul style="list-style-type: none"> <li>- Phytoextraction</li> <li>- Phytostabilisation</li> <li>- Phytodegradation</li> <li>- Phytotransformation</li> <li>- Phytovolatilisation</li> </ul>
Containment	<ul style="list-style-type: none"> <li>- Fast</li> <li>- Cheap</li> </ul>	<ul style="list-style-type: none"> <li>- Potential leakage</li> <li>- Does not reduce contaminant volume or toxicity</li> </ul>	<ul style="list-style-type: none"> <li>- Encapsulation</li> <li>- Capping</li> </ul>
Physical treatment	<ul style="list-style-type: none"> <li>- Comprehensive</li> </ul>	<ul style="list-style-type: none"> <li>- Loss of soil characteristics</li> <li>- Costs</li> <li>- Contaminants have to be discarded or remediated</li> </ul>	<ul style="list-style-type: none"> <li>- Heat treatment</li> <li>- Soil washing</li> <li>- <i>In situ</i> flushing</li> <li>- Air sparging</li> <li>- Soil vapour extraction</li> <li>- Solidification</li> <li>- Vitrification</li> </ul>
Chemical treatment	<ul style="list-style-type: none"> <li>- Cheap</li> <li>- Easy</li> </ul>	<ul style="list-style-type: none"> <li>- Loss of soil characteristics</li> <li>- Contaminants have to be discarded or remediated</li> </ul>	<ul style="list-style-type: none"> <li>- Liming</li> <li>- <i>In situ</i> oxidation</li> <li>- Permeable reactive barriers</li> <li>- Chemical stabilisation</li> </ul>

Selecting the best treatment method depends on the type and quantity of the contaminants, treatment costs, the soil type, and the environmental conditions on the site, among other things (Khan et al., 2004; US DOD 1994). Before selecting the best available method or methods, it is necessary to conduct a thorough investigation of the properties of the soil. This is especially true for *in situ* -remediation, because performance of the remediation process is more difficult to monitor and control during treatment than it is in traditional *ex situ* -remediation processes (Jeltsch, 1990; Morgan and Watkinson, 1992). Among the important data requirements are e.g. particle size distribution, soil homogeneity, permeability, pH, moisture, and redox-potential of the site (US DOD, 1994). Sites are rarely contaminated with just one type of contaminant. Therefore, it is common to use several treatment methods at the same time or in series, as a treatment train (Khan et al., 2004; US DOD, 1994). Soil characteristics frequently limit the range of suitable techniques, but certain properties, such as pH and moisture content, can sometimes be

adjusted. Some characteristics, such as permeability of the soil, may hinder the use of certain remediation techniques. (Penttinen 2001; U.S. DOD 1994)

*In situ* –remediation is a good option on many occasions. Excavating the soil may be too expensive or even impossible. If the area to be treated is very large or the contaminated zone lies deep in soil, it is costly to excavate the whole mass and transport it for remediation. If the contaminated site has buildings or is otherwise in active use, excavation is not an option (Carberry and Wik, 2001). However, challenges do exist with *in situ* -treatments. The time required for the remediation is often considerably longer than with *ex situ* –treatments. Consequently, if time is of essence, *in situ* -treatments may be unsuitable. A major challenge with all *in situ* -treatment methods is to achieve uniform remediation throughout the treatment area. As contaminated soils are usually heterogeneous, a uniform delivery of chemicals to the whole area is very difficult to achieve (FRTR, 2000).

Several *in situ* -methods to treat hydrocarbon-contaminated soils exist, such as air sparging, soil flushing, chemical treatments, reactive walls or zones, stabilisation, and containment (FRTR, 2000; US DOD, 1994). Figure 1 presents general schematics of a few treatment methods. Most of these have the common problem of being dependent on soil permeability. In Finland, most of the soil is glacial till, moraine. The low permeability of moraine hinders the use of many *in situ* -remediation methods. The soils should preferably be quite coarse-grained and homogeneous, otherwise preferential paths form through which the fluid or air will travel leaving parts of the soil untouched. It is, therefore, evident that efficient *in situ* –remediation methods are required, which function well in low-permeability soils.



**Figure 1** General schematics of various treatment methods. From top to bottom: Air sparging, in situ flushing, chemical treatment (injection), and treatment wall (adapted from NFESC, 1997; US EPA, 1996; Ott, 2000; Vidic and Pohland, 1996, respectively).

### 1.2.3. Specific challenges of *in situ* –bioremediation

Bioremediation techniques have usually been thoroughly tested in laboratories with good results, but the transfer to the field has often been quite difficult (for a review, see Allard and Neilson, 1997; Sturman et al., 1995). In laboratories, the tests are often performed under optimal conditions, which do not correlate well with conditions in the field (Davis et al., 2003; Goltz et al., 2001; Mandelbaum et al., 1997; Sturman et al., 1995). Another major factor is the insufficient understanding of parameters concerning scale-up of the techniques. A number of phenomena connected with bioremediation may be scale-dependent, for example advective and dispersive transport processes, microbial population dynamics and influence, sorption processes, and spatial heterogeneity. Observations from the micro- and mesoscale do not always apply at the macroscale, i.e. the field (Davis et al., 2003; Goltz et al., 2001; Schirmer et al., 2000; Sturman et al., 1995). Application of data from laboratory experiments to the field has often lead to unsatisfactory results, e.g. the half-lives of compounds in the field tend to be 4-10 times longer than in the laboratory (Schirmer et al., 2000; Sturman et al., 1995).

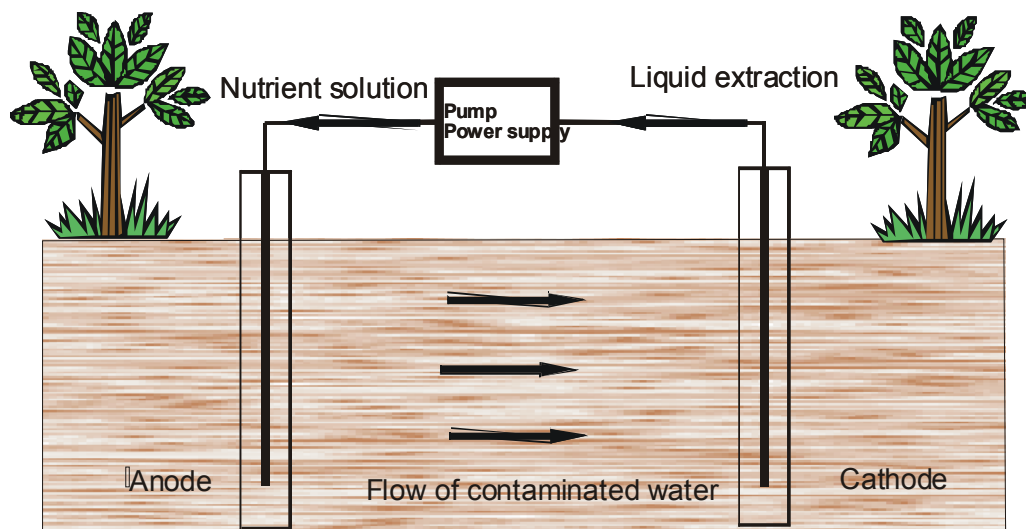
For the biodegradation process to proceed, the contaminant, nutrients, and the microorganisms have to be located at the same place at the same time, and the bioavailability of the contaminant and nutrients have to be adequate for sufficient bioremediation. These prerequisites are easy to fulfil in the laboratory but not in the field (Gierke and Powers, 1997; Sturman et al., 1995). Three major problems are recognised in the scale-up of *in situ* -bioremediation processes: limitations in both transport and biotransformation processes and spatial heterogeneity (Goltz et al., 2001; Schirmer et al., 2000; Sturman et al., 1995). The transfer from laboratory to the field is difficult, because transport rates are often the limiting ones in field scale and most of them are scale dependent. Furthermore, more phases usually exist in the full-scale system than in the laboratory creating additional mass transfer limitations (Schirmer et al., 2000; Sturman et al., 1995). Field conditions, such as temperature and pH, are rarely optimal for the activity of microorganisms and, therefore, limit the rate of biotransformation processes. Geological conditions may also vary considerably within the site: Geological and other spatial heterogeneities are more a rule than an exception at almost every site (Gierke and Powers, 1997; Schirmer et al., 2000; Sturman et al., 1995).

### 1.2.4. Electroremediation

Electrokinetic remediation has traditionally been used to remove metals and polar organic compounds from soils, sludges, and sediments (for a review, see Page and Page, 2002; Virkutyte et al., 2002). Electrokinetic remediation methods use electrodes with a low-level direct current electric field (usually  $<10$  V/cm or mA/cm<sup>2</sup>) installed into the contaminated soil (Acar and Alshawabkeh, 1993; van Cauwenberghe, 1997; Pamucku, 1997). The current mobilises and transports charged chemicals in the soil's liquid phase towards the electrodes. Negatively charged anions and organic compounds will move to the anode, whereas positively charged chemicals, such as metals, will move towards the cathode (van

Cauwenberghe, 1997; Penttinen, 2001). Contaminants can be removed from the electrodes with several methods including electroplating, adsorption onto the electrodes, pumping near the electrodes, precipitation or coprecipitation at the electrodes, complexing, and capturing the contaminants in reactive permeable barriers (Acar and Alshawabkeh, 1993; van Cauwenberghe, 1997; Pamucku, 1997; Virkutyte et al., 2002). Surfactants and complexing agents can be added to increase the solubility or mobility of the contaminants (van Cauwenberghe, 1997)

The main phenomena affecting the movement of contaminants in *in situ* -electrokinetic remediation are electromigration and electro-osmosis. In electromigration, the chemicals move towards the electrodes according to their charges, whereas in electro-osmosis, they are transported by water to the cathode. Water can also transport uncharged organic and inorganic compounds. Electromigration is independent of the pore size of the soil and is, therefore, applicable to both coarse- and fine-grained soils. Electro-osmosis, however, is ineffective in coarse-grained soils (Acar and Alshawabkeh, 1993; van Cauwenberghe, 1997; Pamucku, 1997; Probst and Hicks, 1993; Virkutyte et al., 2002). *In situ* -electrokinetic remediation is suitable for various types of soil if only the moisture content is high enough. The effectiveness of the method decreases considerably if soil moisture content is less than 10% (van Cauwenberghe, 1997; Penttinen, 2001). Direct current creates a pH-gradient in the soil: Electrolysis reactions at the electrodes generate an alkalic zone near the cathode and an acidic one near the anode. A high cation exchange capacity of the soil and the use of suitable buffers will reduce the pH-gradient. (Probst and Hicks, 1993). Figure 2 presents a general schematic of the electro-osmotic remediation technique.



**Figure 2** A simplified schematic of *in situ* -electroosmotic remediation.

Electrodes can be installed either vertically or horizontally in directionally drilled tunnels, or in trenches surrounding the contaminated site (Lageman, 1993; Virkutyte et al., 2002). Metal objects, objects of insulating material, or ore deposits may affect the process; therefore, an investigation of site-specific properties must be performed prior to application (van Cauwenberghe, 1997; Penttinen, 2001). A significant advantage of *in situ* -electrokinetic remediation is the level of control achieved for the direction of the flow. In soils with low hydraulic permeability, electro-osmosis is relatively independent of the pore size permitting a more uniform flow distribution than in pressurised flows that are subjected to channelling. This makes electro-osmosis very useful in heterogeneous, low-permeability soils (Probstein and Hicks, 1993; Shapiro and Probstein, 1993). Another advantage of the technique is that it enables simultaneous *in situ* -treatment of both inorganic and organic contaminants (Pamucku, 1997; Penttinen, 2001). Various kinds of process solutions may be added to the soil to increase the solubility of the contaminants, or to complex or chelate them (Pamucku, 1997). Electrokinetic remediation can be used in combination with other remediation techniques, such as biodegradation and soil vapour extraction. Additionally, electric fields can be used to prevent contaminants from spreading to uncontaminated areas (Lageman, 1993).

Metals are usually present in soils as cations, therefore migrating to the cathode (Page and Page, 2002). This migration is further enhanced by the electroosmotic flow. A number of studies have presented the feasibility of removing metals from soils with electrokinetics. The most common contaminant metals studied are lead (Acar and Alshawabkeh, 1996; Kim et al., 2001; Ottosen et al., 2001; Reed et al., 1995), cadmium (Kim et al., 2001; O'Connor et al., 2003; Reddy and Chinthamreddy, 1999; Vengris et al., 2001), copper (Hansen et al., 1997; Maini et al., 2000a, 2000b; O'Connor et al., 2003; Ottosen et al., 2001), chromium (Hansen et al., 1997; Pamucku et al., 2004; Reddy and Chinthamreddy, 1999), and zinc (Hansen et al., 1997; Maini et al., 2000b; Ottosen et al., 2001; Vengris et al., 2001).

In recent years, the focus of electroremediation has been changing from only heavy metals to including organic contaminants. This is mainly due to the growing understanding of the level and hazardousness of organic contamination in the environment. Although electroremediation of organics is not as widespread as treating metals, positive results have encouraged its implementation (Acar et al., 1992; Ribeiro et al., 2005; Shapiro and Probstein 1993). The contaminants studied include for example phenol (e.g. Acar et al., 1992; Shapiro and Probstein 1993; Yang and Long, 1999), acetic acid (e.g. Shapiro and Probstein 1993), atrazine (e.g. Ribeiro et al., 2005), PAHs (e.g. Maini et al., 2000b), and 2,4-dichlorephenoxyacetic acid (Jackman et al., 2001). Electroremediation of organics is based on electro-osmosis. Electro-osmotic movement can force contaminants from small pore spaces where they might be trapped, electricity provides heat to the ground increasing the solubility of most organic contaminants to the soil water, and heat enhances the activity of the soil microorganisms resulting in a more active degradation of contaminants. Organic contaminants are usually biodegradable; therefore, electrokinetic methods can be used to enhance bioremediation. Spreading nutrients to soils has been tested to remediate soils contaminated with organics (Acar et



al., 1996; Elektorowicz and Boeva, 1996; Reddy et al., 2003; Thevanayagam and Rishindran, 1998). In *certain situations*, however, indigenous microorganisms may not be adapted to the contamination or the density of degrading organisms is too low. Hence, bioaugmentation could be advantageous. A few studies have concentrated on the transport of bacteria by electrokinetics (DeFlaun and Condee, 1997; Lee and Lee, 2001; Wick et al., 2004). They investigated the feasibility to mobilise bacteria in models simulating aquifers with good results. Experiments above the groundwater table are required, however, to ensure an effective remediation of contaminated soils. Jackman et al. (2001) adopted an alternative approach. In their study, they mobilised the contaminant, 2,4-dichloroacetic acid, into the biodegradative zone.

The vast majority of the experiments have been conducted in optimised conditions with homogenised soil and artificially contaminated (spiked) soil. Laboratory experiments with natural, contaminated soils are necessary because heterogeneity of the soil and contaminants affect the performance of the method significantly. In addition, in field experiments a number of problems arise at the field that are not of concern in the laboratory, such as conducting or resistive objects in the ground (Lageman, 1993). The focus of the field experiments has been on remediation of metal-contaminated soils. Table 2 presents key experiences of field tests and laboratory tests with natural, contaminated soils.

**Table 2.** Key references for removal of contaminants from natural, contaminated soils by electrokinetics.

Contaminants and concentrations (mg/kg)	Soil characteristics	Duration	Results: reduction-%, final concentrations	Energy consumption	Reference
<b>Laboratory tests:</b>					
Cr (330), Cu (1 400)	Loamy sand	54 days	Cr <100 mg/kg, Cu 200 mg/kg, average		Hansen <i>et al.</i> , 1997
Hg (685)	Sand	77 days	520 mg/kg, average		Hansen <i>et al.</i> , 1997
Cd (22) Pb (1438)		4 days	Cd 67.6 Pb 49.8%	197 kWh/ton	Kim <i>et al.</i> , 2001
e.g PAH (720)		22 days	< 10 mg/kg		Maini <i>et al.</i> , 2000b
<b>Field trials:</b>					
Cu (300– 5 000), Pb (500–1 000)	Peat soil and sludge	43 days	Cu: 80% Pb 70%	65 kWh/ton	Lageman, 1993
Zn (2 410, average)	Clay	8 weeks	1620 mg/kg, average	160 kWh/ton	Lageman, 1993
As (400 – 500)	Heavy clay	65 days	< 30 mg/kg		Lageman, 1993
Cd (2-3 400)	Landfill, sludge	2 years	< 1-40 mg/kg		Lageman, 1993
Trichloroethylene (1– 507)	Gravel, clay, sandy clay loam	4 months	98.4% reduction 1.1 mg/kg		Ho <i>et al.</i> , 1999a
Trichloroethylene (1– 1 500)	Gravel, clay, sandy clay loam	1 year	< 0.1 – 106 mg/kg		Ho <i>et al.</i> , 1999b
Cr (180-1 100), Cd (5 – 20)		6 months	78% of the area treated below natural background levels of Cr, 70% of Cd	200 kWh/m <sup>3</sup>	Gent <i>et al.</i> , 2004

### 1.2.5. Remediation of oil-contaminated solid and water surfaces

Small-scale oil spills are common in various facilities such as factories and service stations as well as at sea. Approximately 700 000 tonnes of oil from anthropogenic sources end up in the seas annually (NAS, 2003). These are sometimes large-scale

accidents, in which oil tankers release vast amounts of oil. More common, however, are oil spills in connection with ships emptying their bilge water reservoirs into the sea. It is often possible to pump the oil from the soil or the sea in case of large-scale oil spills, but not in minor-scale accidents. In these cases, various sorbents are commonly used to absorb the spilled oil - if anything is done at all.

In case of a larger oil spill at sea, the current practise is to restrict the spreading of oil with booms and to collect it with vessels fitted with oil recovery brushes (Prince, 1997). If the oil reaches the shore, it is recovered mostly mechanically with special machinery or manually with shovels, which is very laborious and often ineffective. On rocky shores, oil can be washed back to the sea, but some oil always remains (Prince, 1997). Sorbents can be used to collect the residual oil from shores or from the surface of water.

#### **1.2.6. Sorbents**

Oil sorbents are an attractive option for oil spill treatment because they allow a fast and complete removal of oil from the surface of water (Adebajo et al., 2003). An oil sorbent should have a number of properties: it should be highly oleophilic and hydrophobic; the absorption rate of oil should be high to enable fast sorption of oil and to prevent the spreading of oil; and it should be buoyant and resilient in water (Choi and Cloud, 1992; Deschamps et al., 2003; Teas et al., 2001). The sorbents in use today can be classified as polymers, natural materials, or treated cellulosic materials (Deschamps et al., 2003). Several studies of various natural, synthetic, and mineral sorbents exist (e.g. Choi and Cloud, 1992; Deschamps et al., 2003; Reynolds et al., 2001; Teas et al., 2001; Toyoda and Inagaki, 2003). The majority of commonly used commercial sorbents are synthetic and made of polypropylene or polyurethane (Teas et al., 2001). These have good hydrophobic and oleophilic properties but their non-biodegradability is a major disadvantage. The only practical option to dispose of the used oily sorbents is burning but incineration is an expensive treatment method (Choi and Cloud, 1992; Deschamps et al., 2003). If the material were biodegradable, another option would be to treat the oily sorbents with compost-like techniques similar to treating oil-contaminated soils. However, in cold climate areas, biodegradation of oil can be slow in natural environments, especially in environments that do not favour a high indigenous microbial numbers.

A number of natural sorbents have been studied for use in oil spill cleanup: e.g. cotton wool, bark, milkweed, kapok, kenaf, rice straw, even dried biomass of an aquaphyte (Table 3). Most of them have higher absorption capacities than synthetic ones but they often absorb water well, which is a disadvantage when used in marine environments (Choi and Cloud, 1992; Wei et al., 2003). Occasionally their hydrophobicity can be enhanced by various treatments; however, that increases the overall costs of the sorbents.

**Table 3.** Oil absorption capacities of various materials from the surface of water and their key references.

Sorbent material	Contaminant	Absorption capacity (g oil / g sorbent)	Reference
Cotton	Light crude oil petroleum	~ 35 26	Choi and Cloud, 1992 Deschamps <i>et al.</i> , 2003
Kenaf	Light crude oil	~ 8	Choi and Cloud, 1992
Milkweed	Light crude oil	~ 40	Choi and Cloud, 1992
Wool	Diesel Crude oil	13 12	Radetic <i>et al.</i> , 2003 Radetic <i>et al.</i> , 2003
Kapok	Machine oil	40	Hori <i>et al.</i> , 2000
Bark	Bunker A (without water)	13.4	Saito <i>et al.</i> , 2003
Acetylated rice straw	Machine oil	16.8-24	Sun <i>et al.</i> , 2002
Aquaphyte biomass	Crude oil	4.8	Ribeiro <i>et al.</i> , 2000
Polypropylene	Light crude oil	10	Choi, 1996
Expanded perlite	Heavy crude	3.25	Teas <i>et al.</i> , 2001
Exfoliated graphite	Crude oil	80	Toyoda and Inagaki, 2003

### 1.2.7. Cotton grass

When peat is excavated from bogs, it is purified by separating other components. One major component is cotton grass fibre (Fig. 3). Sheathed cottonsedge, cotton grass (*Eriophorum vaginatum*), is a common plant in peat bogs. Cotton grass fibre is formed when the plant dies and partly degrades detaching the plant fibres in a slatted form. Because of the acidic and anaerobic nature of the bog, the stem and root of the plant do not decompose totally, but deteriorate into fibre that has resisted degradation for hundreds of years. In aerobic conditions, however, cotton grass fibres can be biodegraded. In Finland, the production volume of cotton grass is a few hundred cubic metres a year

(Määttä, 2003). Only a small portion of this by-product of peat excavation has some practical use, for example in the textile industry; most of the cotton grass fibre remains unutilised.



**Figure 3** *Cotton grass (Eriophorum vaginatum) and cotton grass fibre. Photo by Misty Nikula-Ohlsen (TREC 2004), Courtesy of ARCUS.*

## 2. Aims of the thesis

Innovative, new techniques are required for cleaning up hydrocarbon-contaminated environments. *In situ* -bioremediation methods are suitable for various situations, but in cold climate areas, their effectiveness is usually hindered by low temperature. Another challenge to overcome in many *in situ* –remediation techniques is to achieve a uniform distribution of fluids across the area. In bioaugmentation or biostimulation, a uniform distribution of nutrients and microorganisms is required, which is very difficult with present technology in low-permeability soils.

Sorbents are practical solutions for small-scale oil spills on solid or water surfaces. Most sorbents are synthetic, however, and can not be disposed of using ecologically sound methods. Some natural sorbents exist, but they are not suitable for absorption on the surface of water, because they absorb water as well. Biodegradable sorbents could be disposed of in compost-like systems. However, in environments lacking a high initial density of degrading microorganisms, degradation of oil from the sorbents might be slow.

The aim of this thesis was to develop more efficient, cost-effective, and ecologically sound techniques to treat oil spills on water or on solid surfaces and underground contamination, especially in cold climate areas. The specific aims were:

- 1) To examine whether dispersing bacteria in soils is feasible with electro-osmosis;
- 2) To assess whether enhancing bioremediation with electrokinetics would result in efficient degradation of creosote in an old wood impregnation site;
- 3) To assess the suitability of cotton grass fibres as an oil sorbent for collecting oil spills on water surfaces;
- 4) To examine whether dried microbial suspension on a biodegradable peat-cellulose oil sorbent would enhance the degradation of oil from the oily sorbents in compost-like systems.

The overall aim was to develop practical solutions for treating hydrocarbon contamination in cold climate areas.

### **3. Materials and Methods**

A general overview of the materials and methods is given below; detailed descriptions of the experimental designs and analyses have been given in the respective papers (I-IV).

#### **3.1. Materials**

The cotton grass fibres used in the experiments were received from VAPO Ltd, Finland, and the peat and peat-cellulose fabrics were received from Ahlström Ltd, Finland. The soil used in laboratory tests of creosote-contaminated soil was obtained from the pilot-test site located in Mikkeli, in the eastern part of Finland.

Unprocessed cotton grass fibres, purified fibres, and sorbent mats produced either with or without a binding agent were used in absorption experiments (Paper III). The synthetic sorbent in Paper III was an Oil-Only sorbent (Unisorb) and the one in Paper IV was from an oil collection kit (Mobil Oy). Whatman filter paper no. 3 served as a cellulose control (Paper IV). Several types of oil were tested in absorption experiments with cotton grass (Table 2, Paper III). Synthetic motor oil (Mobil Oil SAE 0W-40) was used in experiments on microbially supplemented peat-cellulose fabrics.

The bacterium used in the mobilisation tests (Paper I) was a phenol-degrading, flagellar *Pseudomonas fluorescens* –strain CF 600.1:856 GFP (green fluorescent protein) Rif (rifampicin) (Sarand et al., 2001).

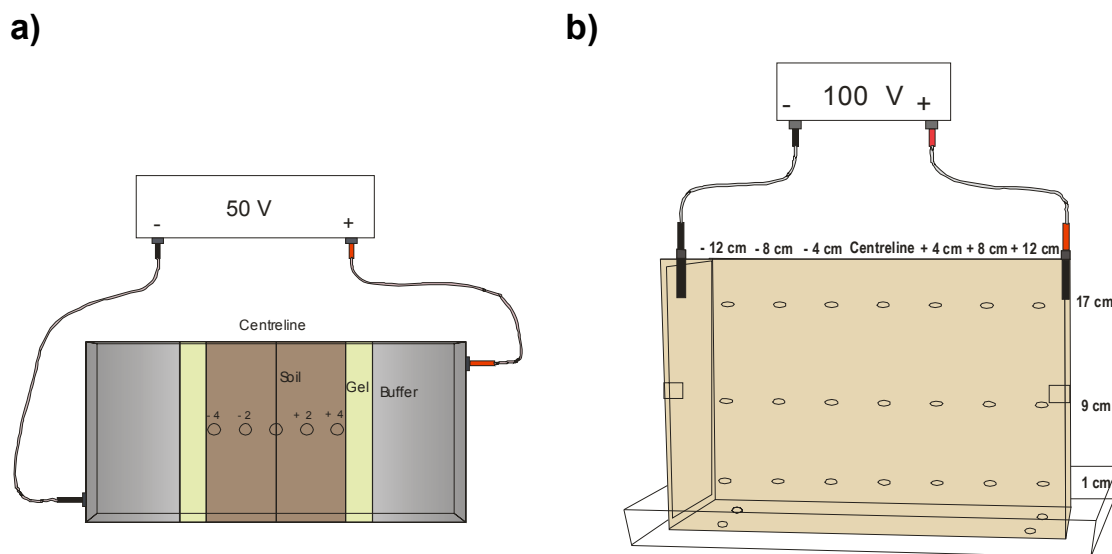
#### **3.2. Experimental set-ups**

##### **3.2.1. Mobilisation tests (Paper I)**

Gel-electrophoresis set-ups and microcosms were used to determine whether electro-osmosis can be used to disseminate bacteria in soils to facilitate bioaugmentation. Garden soil, fine sand, and clay were used as test soils.

In the gel electrophoresis device, an agarose gel was cut from the middle leaving only small strips (ca. 1,5 cm wide) at both ends, and the empty space in the middle was filled with soil (Fig. 4a). With each soil, an electro-osmosis test was performed by applying a direct current and a control test without electricity. The microcosm tests were performed in specifically designed microcosms with electrodes at both ends mimicking set-ups used in field applications (Fig. 4b). The inoculum was spread vertically to the centreline of the soil in the microcosm tests and horizontally in the bacterial migration velocity tests.

Samples were taken from several points in the soil to monitor the distribution of the bacteria and to determine pH values and moisture content.

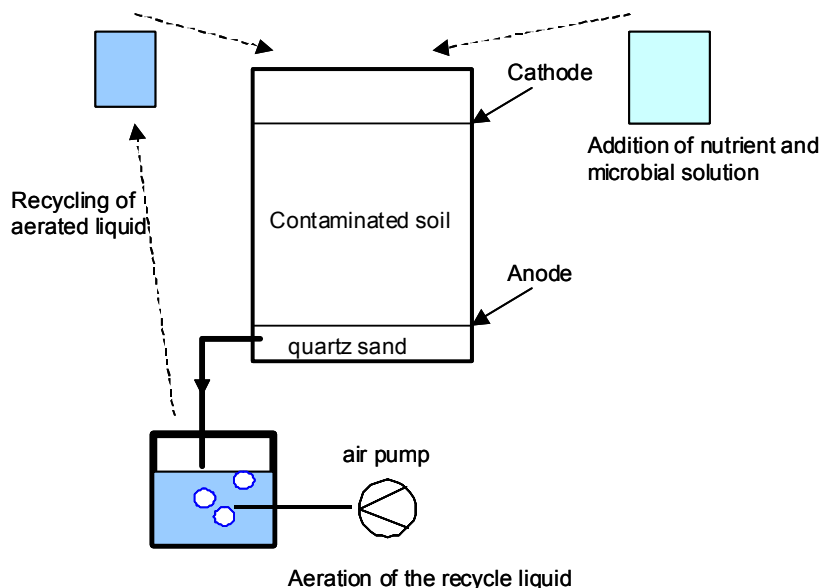


**Figure 4** *Schematic illustration of the mobilisation test set-ups. (a) Gel electrophoresis set-up and sampling points in the bacterial migration velocity tests. (b) Microcosms used in the tests. The cosm stands in water and the soil absorbs water through the holes in the bottom. The sampling points are also shown (Fig. 1 in Paper I).*

### 3.2.2. Laboratory and field biodegradation studies of creosote-contaminated soil (Paper II)

The laboratory tests were performed in 20-l insulated boxes (cool boxes) with steel wire mesh electrodes, cathode at the bottom and anode at the top (Fig. 5). At the bottom of the reactors, under the cathode, was a 2-cm layer of quartz sand and a small pipe, through which outcoming liquid could be collected by pumping or by gravity. The reactors contained approximately 15 l (23-26) kg of soil. The voltage was 5 V/cm. Samples were taken from the soil and analysed for PAH-concentration, viable count of bacteria, pH, and moisture content.





**Figure 5** *Experimental set-up design for tests combining bioremediation and electroremediation (Fig. 1 in Paper II).*

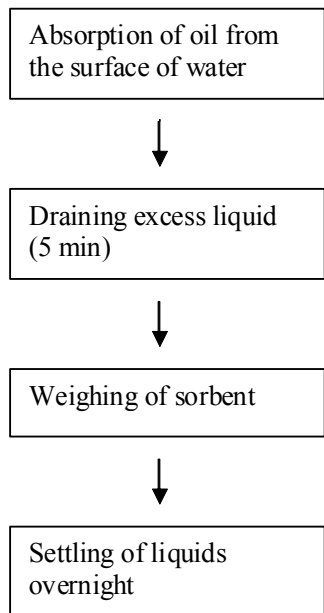
The field set-up consisted of a cathode installed vertically in the middle of the test area and six anodes installed vertically to form a circle around the cathode with a radius of 2.5 m. The electrodes and wells were later repositioned horizontally to ensure a more even dissemination. Injection wells were installed beside the anodes and an extraction well beside the cathode. The anodes and the cathode were attached to a transformer with a constant voltage of 270 V throughout the trial. Water pumped from the groundwater well was piped to nearby treatment equipment, in which water was first run through an oil separator to separate pure creosote and then treated in a biological, aerated carrier matrix reactor with added nutrients. The water was also run through an activated carbon filter before circulating back to the soil.

### 3.2.3. Absorption tests of cotton grass (Paper III)

Absorption capacities and rates were measured by hanging a piece of sorbent, on a coarse steel wire mesh, from a scale above a dish containing oil. The dish was slowly raised with a motorised unit until there was contact between the oil and the sorbent (Tavisto et al., 2003). The weight of the absorbed oil was measured every second.

Absorption capacities of cotton grass fibres from the surface of water were measured by applying a weighed piece of sorbent to a known amount of oil floating on the surface of 100 ml of water in a beaker. The sorbent was drained of excess liquid and weighed. The remaining liquid in the beaker was poured into a measuring cylinder to settle over night in

order to examine whether the sorbent had absorbed water together with oil. A schematic diagram of the protocol is presented in Figure 6.



**Figure 6** *A schematic diagram of the protocol in oil absorption tests from the surface of water.*

#### **3.2.4. Microbially supplemented oil sorbent (Paper IV)**

The microorganisms were enriched from the soil of an old service station area. M9-mineral medium was used for enrichment. Motor oil served as the sole growth substrate. The microbially supplemented sorbent was produced by drying microbial suspension into a peat-cellulose fabric. After sufficient growth, a piece of peat-cellulose fabric (test cloth) was submersed in the microbial suspension and let to dry. The dry fabric was cut in smaller pieces and used as an inoculum for subsequent cultivation. After several cycles, the suspension was absorbed to six pieces of fabric, which were then air-dried. For the degradation tests, 30 microbially supplemented cloths and 30 clean cloths were soaked in motor oil and placed in boxes containing either garden soil or sand. The incubations lasted for 1, 3, 6, and 12 weeks, after which the remaining oil was extracted from the cloths.

### 3.3. Hydrocarbon analyses

Dichloromethane extraction and gravimetric measurements were used to determine the oil concentration in experiments with oil sorbents (Papers III and IV). Toluene extraction (Karstensen, 1996) and a gas chromatograph with a flame ionisation detector or a mass spectrometry detector were used in creosote degradation tests (Paper II). A summary of the experimental set-ups and analyses is presented in Table 4.

**Table 4.** *A summary of the set-ups and analyses of the experiments.*

	<b>Bacterial mobilisation tests</b>	<b>Electrokinetically enhanced biodegradation of creosote</b>	<b>Absorption tests of cotton grass</b>	<b>Microbially supplemented oil sorbent</b>
Set-ups, Experiments	Gel electrophoresis, microcosms	Insulated boxes, field test	Absorption tests from oil (scale test), from the surface of water (beakers)	Biodegradation tests in garden soil and sand
Analyses	Viable count of bacteria, pH, moisture	PAH-concentration (gas chromatograph), Viable count of microbes, pH, moisture	Gravimetric measurement of oil absorption capacity	Oil concentration (gravimetric), viable count of microbes

### 3.4. Remarks on methodology

When dealing with heterogeneous material, such as natural soil, it is challenging to obtain a representative sample. It is especially difficult if the soil should remain as intact as possible during the experiment. Contamination in soils is rarely, if ever, spread homogeneously across the area: Relatively contaminant-free zones may exist in soils and sampling from that area only gives the impression that the soil is already clean. On the other hand, sampling from hot spots, where contamination concentration is markedly higher than on average, gives misleading results as well. In the diesel degradation experiments, sampling quite likely played a large role. Although taken from various parts of the soil, the total volume of the samples was quite small because of an effort to preserve the soil. This difficulty in obtaining a representative sample most likely explains the great variation seen in the samples.

## 4. Results

This section presents a summary of the results. Detailed results have been presented in the original papers. Papers I and II discuss the possibility of enhancing bioremediation of hydrocarbon-contaminated soils with electrokinetics. Papers III and IV focus on the use of oil sorbents for treating oil-spills at land and at surface waters.

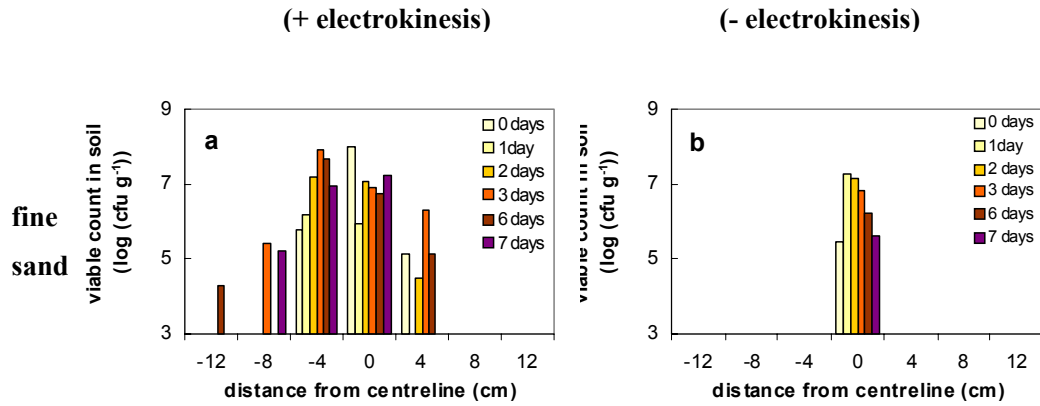
### 4.1. Electrokinetically enhanced bioremediation (Papers I and II)

#### 4.1.1. Mobilisation tests (Paper I)

Paper I presents the experiments to disseminate *Pseudomonas fluorescens* –strain through various soil types with electro-osmosis to facilitate bioaugmentation in *in situ* - remediation. The gel-electrophoresis set up simulated conditions below the ground water table and 3-l microcosms simulated field trial situations above the ground water table.

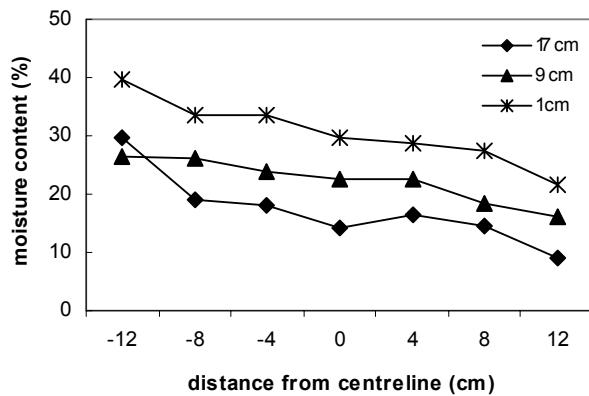
A constant voltage of 2 V cm<sup>-1</sup> was used in the gel electrophoresis tests. In all the tests with electricity, the bacteria moved mainly towards the cathode. In fine sand, the bacteria moved at the following rates (cm/h): 1 in fine sand, 0.6 in garden soil, and 0.1 in clay. After 24 h, turbidity was observable in the buffer solution (water) on the cathode side of the gel in the tests made with fine sand and garden soil resulting probably from bacterial suspension. In tests without electricity, no turbidity was observed nor when no bacteria had been added to the soil.

The microcosm set-up, in which soil “stands” in water, simulates field conditions above the water table. Direct current (constant voltage: 4 V/cm) was applied over the electrodes, and samples were taken at appropriate intervals and plated on Luria agar plates containing cycloheximide and rifampicin. Figure 7 (a-b) shows the viable counts of *Ps. fluorescens* –strain in fine sand with or without electricity. With each soil, the net movement of the bacteria was towards the cathode. The current varied from 3 to 56 mA during the tests. The variation in the current was partly due to the use of phosphate buffers (100 mM K<sub>2</sub>HPO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub>) to control the pH providing the system with current transporting ions. The phosphates (40 ml) were added vertically just beside the electrodes before sampling. In unbuffered preliminary tests, the pH near the anode sank to 2; at the cathode, it eventually rose to near 14. With buffering, the pH values stayed between 5 and 9 - a pH range in which the viability of bacteria is unaffected.



**Figure 7** Viable counts of GFP-labelled *Ps. fluorescens* at different points in microcosm tests. (a) Fine sand with electrokinesis, (b) fine sand without electrokinesis (adapted from Fig. 4 in Paper I).

The moisture gradient varied according to the soil type, being significant only in the densest soil, clay. In garden soil and fine sand, no real moisture gradient was evident. Figure 8 shows the moisture gradient for clay at three different soil depths (1, 9, and 17 cm from the bottom) after 18 days when it reached its maximum. The moisture content is higher at the cathode than at the anode indicating electroosmotic flow of water.

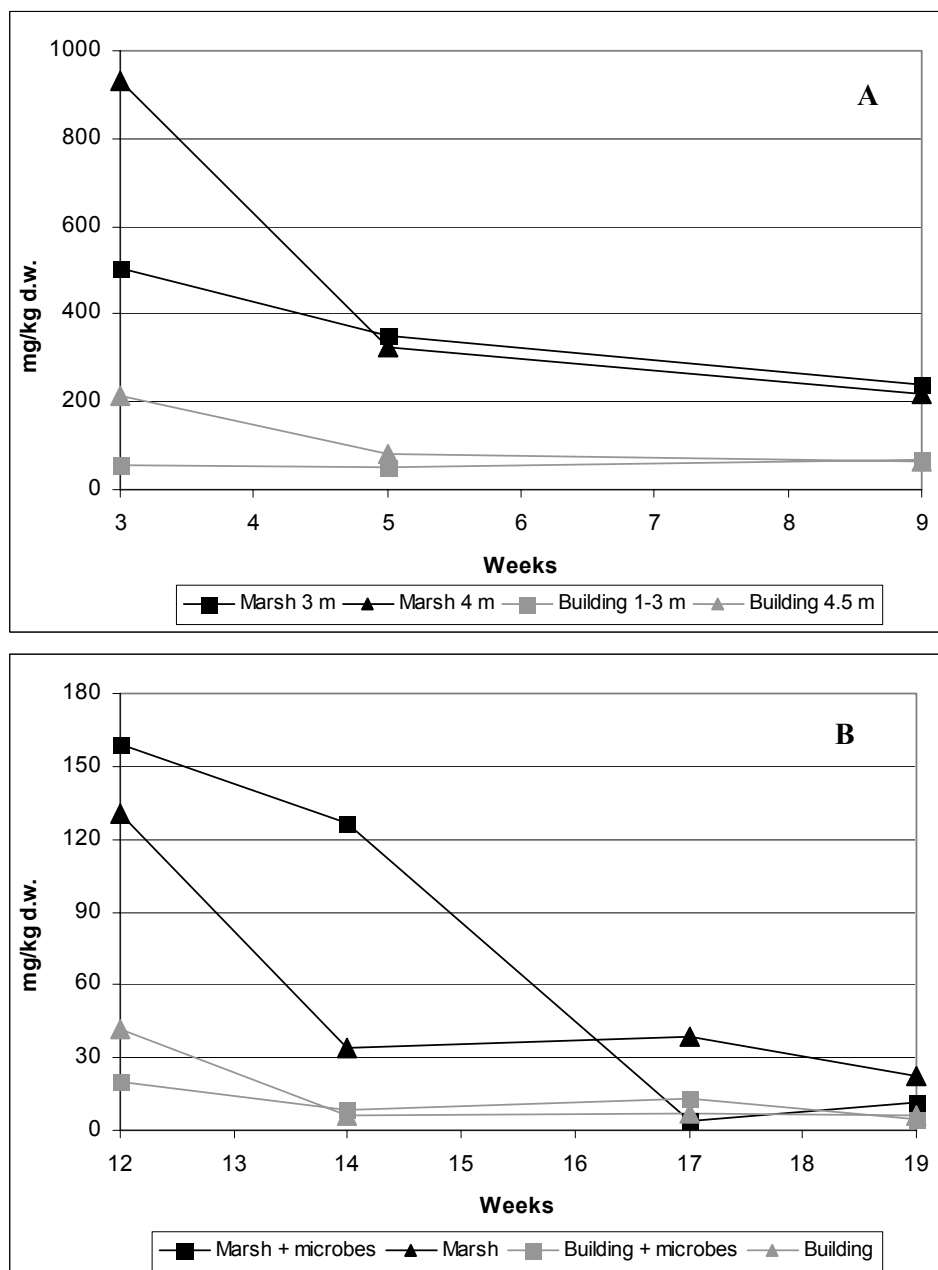


**Figure 8** Moisture gradient formed in clay after 18 days in upright microcosms mimicking conditions above groundwater table. Water sinks by gravitation at the cathode and the horizontal moisture gradient leads to back flow in the free water at the bottom (Fig. 3 in Paper I).

#### **4.1.2. Creosote degradation (Paper II)**

The field test site was a former impregnation plant of railway sleepers contaminated with creosote beyond groundwater table level to the base rock. The laboratory tests were performed with soil samples excavated from two depths near the base of the impregnation plant building and from two depths of a nearby, heavily contaminated marsh. The purpose of the laboratory test was to evaluate the site and the soil conditions in order to assess which approach to take in the upcoming field tests. Therefore, the laboratory test was not set up as a comprehensive stand-alone experiment; each treatment was performed in only one reactor. Furthermore, it was run partly simultaneously with the field test, and when necessary, altered to give answers to field-test questions. The field test was performed on the area next to the impregnation plant.

The reactors were saturated with nutrient solution for two weeks before applying direct current. The reactors in the laboratory tests received 200 ml of nutrient solution (ca. 8 ml/kg of soil) four times a day, five days a week. The outcoming liquid was aerated and recycled back to the reactors. After ten weeks of incubation, the reactors were emptied and filled again, but this time the samples from the marsh (reactors 1 and 2) were combined, mixed, and divided into two reactors (1 and 2). The building samples underwent the same procedure (reactors 3 and 4). To reactors 1 and 3, 200 ml of microbial suspension (ca. 8 ml/kg of soil) from enrichment cultures was added once a week in addition to nutrient solution. During the first seven weeks of incubation with electricity, the PAH-concentrations in the marsh soils (marsh 3 m and marsh 4 m) decreased 68% and in the building soils (building 1-3 m and building 4.5 m) 51% on average (Fig. 9).



**Figure 9** Sums of PAH-concentrations in the soils A) weeks 3-9, B) weeks 12-19 (Fig. 5 in Paper II).

The field pilot test started in September 2005 and continued until the end of December. Nutrients, microorganisms, and oxygen were introduced into the soil (100 m<sup>3</sup>) by electrokinesis and hydraulic pumping. Approximately 50 l (0.5 l/m<sup>3</sup>) of microbial and 600 l (6 l/m<sup>3</sup>) of nutrient solution were added to the water pumped into the ground. During the first seven weeks of treatment, the nutrient- and oxygen-supplemented water was injected

through vertically installed, perforated pipes next to the anodes. During this time, the electric current was approximately 1 A, and the soil temperature 0.5 m from the cathode was approximately 20 °C. To increase the conductivity of the soil, the orientation of the injection pipes was changed to a horizontal position. The current as well as the temperature increased up to 6.5 A and 35 °C, respectively. The temperature sensor outside the treatment area showed a steady temperature of 5 °C throughout the test period. According to the composite samples, total PAH-concentrations decreased by 50-80% in the area during three months of treatment, whereas mineral oil concentrations decreased only by 30%.

**Table 5.** *Average contaminant concentrations of composite samples during the field trial (Adapted from Table 4 in Paper II).*

	C <sub>10</sub> -C <sub>21</sub> mg/kg	C <sub>22</sub> -C <sub>40</sub> mg/kg	Mineral oil mg/kg	PAHs mg/kg	oxy-PAHs ? mg/kg	PAHs (total) mg/kg
Beginning	90	210	300	81	29	110
7 weeks	73	153	227	40	13	53
12 weeks	80	173	213	35	8	42

\* oxygenated forms of PAHs

## 4.2. Oil sorbents (Papers III and IV)

These studies focused on developing a natural, biodegradable sorbent for collecting oil spills on the surface of water (Paper III) and on enhancing the degradation rate of oil from biodegradable oily sorbents (Paper IV).

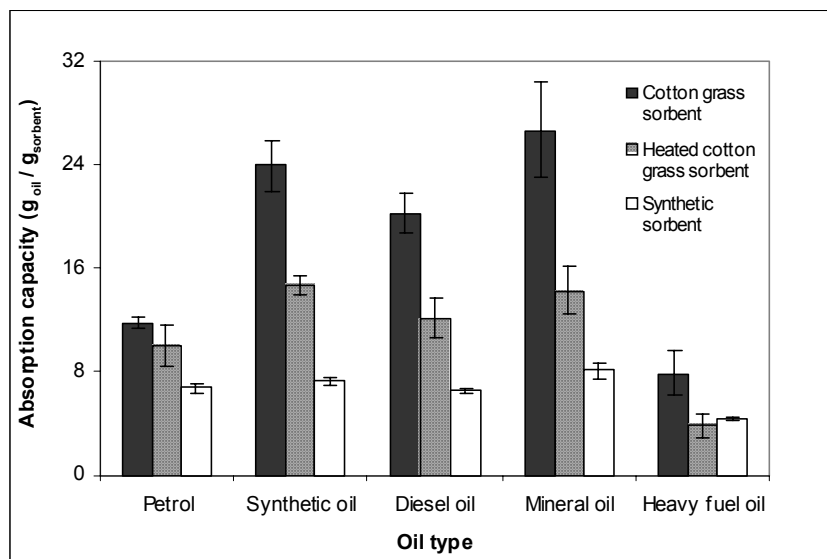
### 4.2.1. Cotton grass as an oil sorbent for surface waters (Paper III)

In preliminary tests, the absorption capacity and rate of several types of cotton grass sorbents (fibres or processed products) were examined and compared with a control, a synthetic, commercially available sorbent intended for sorption of oil spills, Oil-Only (Unisorb). The absorption capacities and rates of these fibres and mats were determined for several common vehicle fuels or engine oils. Each type of cotton grass sorbents absorbed more oil than the synthetic control, up to three times as much oil.

Based on the absorption capacities and rates, the cotton grass sorbent with polyester was selected for further tests. To study the effect of heat treatment on the absorption properties of cotton grass, heated cotton grass sorbent with polyester was chosen as the



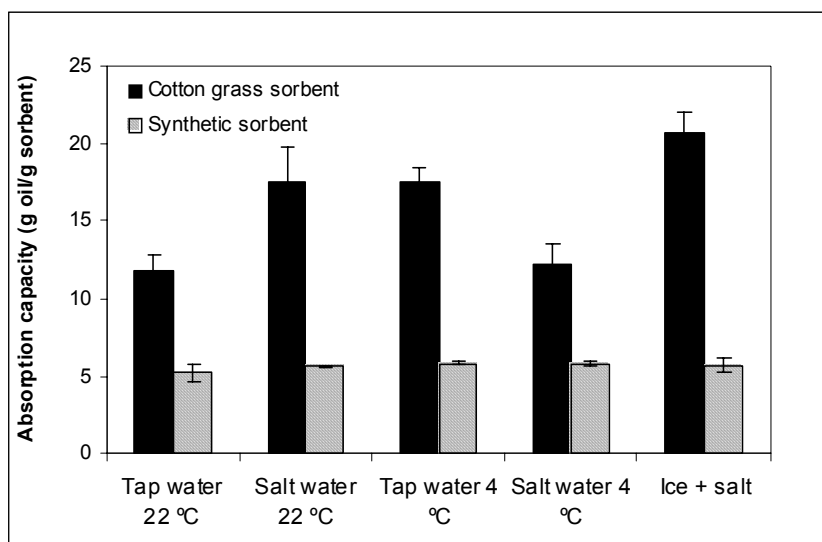
second test sorbent. Cotton grass is suited for absorption on the surface of water because it absorbs very little water. However, when cotton grass was left on water for several days, it wetted and lost some of its absorption capacity (data not shown). Fig. 10 shows the oil-absorption capacities from the surface of water.



**Figure 10** *Absorption capacities from the surface of water. Cotton grass sorbent with polyester, heated cotton grass sorbent with polyester, and the synthetic, Oil-Only sorbent. Absorption capacities are shown as g of absorbed oil per g of sorbent. Error bars depict standard deviation (adapted from Fig. 4 in Paper III).*

Cotton grass sorbent absorbed more oil from the surface of water than the heated cotton grass sorbent or the synthetic sorbent in each case. After leaving the remaining liquid to stand over night in a measuring cylinder to separate water and oil, the exact amount of water was measured. None of the sorbents absorbed water in any of the tests. With heavy fuel oil, this measurement was impossible, however, because the liquids did not form separate layers. The amount of residual diesel oil left on the surface of water was minimal with every type of sorbent and did not increase with increasing amount of oil until they reached their respective maximum absorption capacities. The amount of residual oil after absorption was also determined when the original amount of oil was 90% of the maximum absorption capacity of the cotton grass sorbent. While the residual amount using cotton grass sorbent was 0.02%, the residue with heated cotton grass sorbent was 11.2% and with synthetic sorbent 56.1%.

The effects of low temperature and salt water on the absorption capacity of cotton grass sorbent were determined to assess its performance in conditions prevailing in the Baltic Sea. (Fig. 11, unpublished data).



**Figure 11** *Absorption capacities of diesel oil from the surface of water in two salt concentrations (tap water, 0.7%) and temperatures (4 °C, 22 °C), and from crushed ice (salt water, 4 °C). The salt water represents the average salt concentration of the water in the main basin of the Baltic Sea. Absorption capacities are shown as g of absorbed oil per g of sorbent, error bars depict standard deviation.*

Cotton grass absorbed two to three times as much diesel as the synthetic sorbent. Salt water or cold temperature did not affect the absorption capacity negatively; on the contrary, the lowest absorption values were achieved for tap water at ambient temperature. The highest absorption capacity for cotton grass was achieved with crushed ice on top of cold, salty water. No significant differences existed in the absorption capacity of the synthetic sorbent under varying conditions.

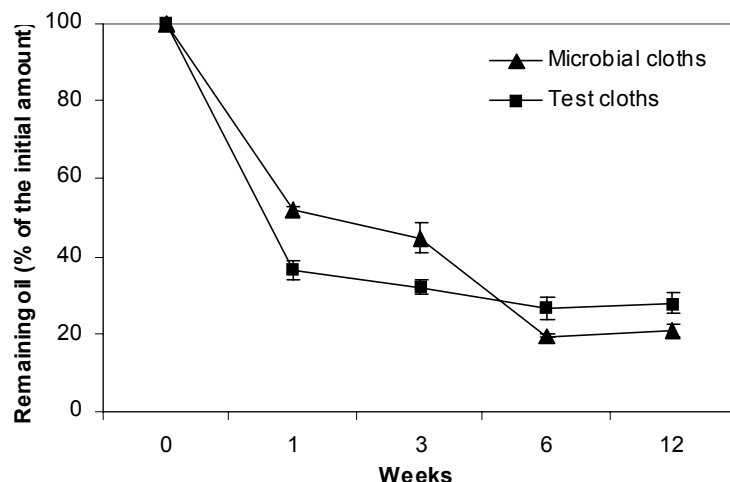
#### 4.2.2. Microbially supplemented oil sorbent (Paper IV)

To examine the effect of microorganisms inoculated on oil sorbent on the degradation rate of oil, a peat-cellulose fabric (test cloth) was selected instead of cotton grass, despite of its lower oil-absorption capacity. This was because the hydrophobicity of cotton grass might retard degradation of oil from the sorbents because biodegradation occurs at the oil-water interface.

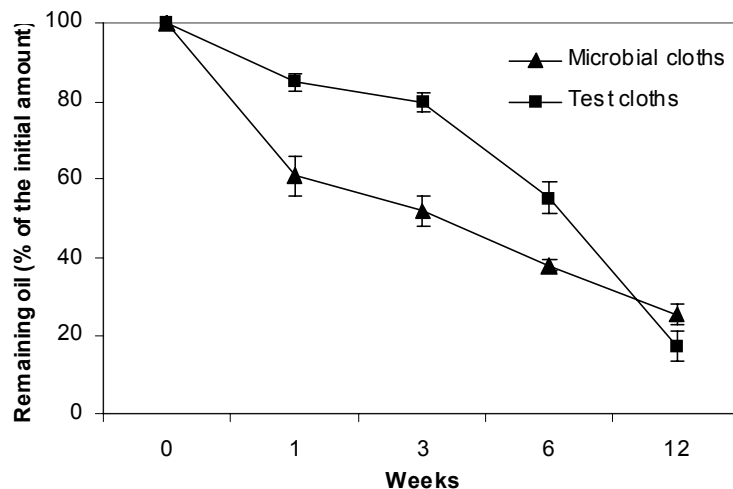
In the biodegradation tests, microbially supplemented and untreated cloths soaked in motor oil were buried in garden soil and sand. The quantity of remaining oil was measured 1, 3, 6, and 12 weeks later (Fig. 12 a and b). The degradation of oil was rapid during the first week in garden soil: 48% of oil had degraded in microbially supplemented cloths and 64% in clean cloths. In sand, 39% of oil had degraded during the first week in the

microbial cloths and 15% in clean cloths. After 12 weeks, no distinctive difference was detectable between microbially supplemented and clean cloth in either soil. The amount of oil leaked from the oil-saturated, microbially supplemented cloths to the immediate vicinity of the cloths in garden soil was less than 10%. In sand, the amount of leaked oil was less than 5%. The respective amounts for the oil-saturated clean cloths were approximately 5% and 2.5%. No oil could be detected elsewhere in the soils.

A)



B)



**Figure 12** Oil concentrations in microbially supplemented cloths and clean cloths during biodegradation tests in A) garden soil, B) sand. Incubation times 1, 3, 6, and 12 weeks. Error bars depict standard deviation (Fig. 2 in Paper IV).

The viable count of microorganisms in the cloths was determined after 1, 3, and 12 weeks of incubation. No significant difference between microbial densities of the microbially supplemented and the clean test cloth was evident in garden soil (Table 6). Viable counts of the microbial cloths in sand and in garden soil were similar almost throughout the whole test as well as that of the clean cloth in garden soil. The viable count of the clean cloth in sand was slightly less than that of the other cloths.

**Table 6.** *Viable counts of microorganisms in microbially supplemented cloths and clean cloths during biodegradation tests in garden soil and in sand after 1, 3, and 12 weeks (Table 1 in Paper IV).*

WEEKS	MICROBIAL CLOTH		TEST CLOTH	
	Garden soil cfu/g	Sand cfu/g	Garden soil cfu/g	Sand cfu/g
1	$6.66 \cdot 10^8$	$1.74 \cdot 10^8$	$3.38 \cdot 10^8$	$3.83 \cdot 10^7$
3	$3.61 \cdot 10^8$	$6.15 \cdot 10^8$	$9.32 \cdot 10^8$	$4.48 \cdot 10^7$
12	$7.32 \cdot 10^8$	$2.65 \cdot 10^7$	$4.77 \cdot 10^8$	$3.47 \cdot 10^7$

In summary, electrokinetic mobilisation of Ps. fluorescenes –strain appeared to be possible in fine sand, garden soil, and in clay. The net movement of the bacterium was towards the cathode in each soil. In laboratory tests with creosote-contaminated soil, the average reduction of PAHs in marsh soils was 68% and in the building soils 51% after seven weeks. In the field pilot test of the creosote-contaminated soil, total PAH-concentrations decreased by 50-80% during 12 weeks. Cotton grass sorbent absorbed two to three times as much oil as the synthetic sorbent from the surface of water, even from water with low salinity and at low temperature. In tests with microbially supplemented oil sorbent, approximately the same amount of oil had degraded from microbially supplemented cloths and from clean cloths after 12 weeks of incubation in garden soil and in sand.

## 5. Discussion

### 5.1. Electrokinetically enhanced bioremediation

The results clearly show that electroosmosis is an effective way to move bacteria through medium- and fine-grained soil. Even though bacteria usually have a negative surface charge in this pH-range and, therefore, might be expected to move electrophoretically towards the anode (Bayer and Sloyer, 1990), the net movement in the tests presented in Paper I was the opposite: Electroosmotic movement was stronger than electrophoretic migration. This bias depended on soil type ranging from clear (clay) to modest (fine sand, garden soil). The cell surface charge of bacteria is assumed to be affected by three groups: proteins, polysaccharides, and hydrocarbon-like compounds (Boonaert and Rouxhet, 2000). Although bacteria usually have a negative net surface charge in soils, electric fields impact several parameters of soil that affect electrokinetic mobility of bacteria, for example pH, soil water content, temperature, and heavy metals (Collins and Stotzky, 1992; Lear et al., 2004). However, low electric currents do not appear to be harmful to the cell-surface properties of bacteria or to microbial communities (Lear et al., 2004; Luo et al., 2005); low currents may even stimulate activity of the bacteria (Jackman et al., 1999).

In clay-microcosm, in which a moisture gradient did form, some bacteria may have sedimented to the water below the soil. As a result, some bacteria may have emerged at distant locations without the aid of electrokinetic transport. Another factor effecting dissemination of bacteria may have been the flagellar motility of that particular strain. It is interesting to note, however, that in tests performed with fine sand without electricity, the strain was reluctant to move anywhere although motile. The bacteria moved considerably slower in clay than in the other soil types. In fine sand, average pore size is clearly large enough for relatively unhindered movement whereas in clay, pore size may become a limiting factor. The fact that bacteria did migrate shows, however, that passage through the pores in clay is indeed possible. Lee and Lee (2001) studied the transportation of a NAPL-degrading *Pseudomonas*-strain in sandy loam in model aquifers. They observed mostly electrophoretic transport of the strain to the anode. However, after 9 h, migration of the bacterial cells to the anode stopped, and after 12 h, most of the viable cells were detected at the loading spot. The viable count of bacteria was very low after 12 h. The loading spot was near the cathode with only one sampling spot on the cathode side. The set-up may have led to the observation of mainly electrophoretic transport of the strain. DeFlaun and Condee (1997) used a model aquifer and obtained transport rates of 1.7-2.4 cm/h and 1.2-4 cm/h for an *Escherichia coli*- and a *Burkholderia cepacia* -strain, respectively, depending on the voltage and the buffer. The inocula were added near the cathode end in the experiments and the bacteria migrated towards the anode. Electroosmotic flow was not to be expected to occur to a great extent due to the relatively high permeability of the soils and the ability of the water level to adjust freely. Wick et al. (2004) detected a 90% bias for electroosmotic movement in a model aquifer with a

*Sphingomonas* sp. Their result is in accordance with the results of the studies presented in Paper I.

The laboratory tests of the creosote degradation showed significantly higher relative degradation in high initial contaminant concentrations than with low initial concentrations. The heavily contaminated marsh soil even reached approximately the same end concentration level than the less contaminated building soil. Therefore, bioremediation appears to be very well suited for cleaning soils with high contaminant concentrations. The total PAH-reduction was over 90% during the laboratory tests. This is in accordance with the studies of Atagana (2004), who reported a 90% reduction of creosote after ten months in a landfarming experiment of heavily creosote-contaminated soil (> 310 000 mg/kg) in South Africa. In the tests presented in Paper II, the differences in PAH-concentrations between samples taken from the top, middle, or bottom part of the reactors were insignificant (data not shown). This suggests that evaporation did not play an important role in the reduction of contaminant concentrations. The outcoming liquid was recycled back to the reactors, which would exclude reduction due to leaching, suggesting that the reduction, indeed, results from microbial activity. This is confirmed by the fact that the PAH- and oil concentrations in the outcoming liquid in laboratory tests were low, both with the relatively mildly contaminated soil from near the building and with the highly polluted marsh soil. This is an important factor considering *in situ* -remediations. During the pilot test, electricity increased the soil temperature to a level suitable for active microbial degradation. Eriksson et al. (2003) discovered that low temperature limited PAH-biodegradation of aerobic enrichment cultures: After 90 days, the PAH-reduction was up to 88% at 20 °C but only 53% at 7 °C. The increase of current and soil temperature in the last few weeks of the field test presented in Paper II was probably due to the installation of horizontal wells instead of vertical ones. Horizontal wells ensured a more effective dissemination of conductive buffer and nutrient solution than vertical wells. Niqui-Arroyo et al. (2006) studied the degradation of phenanthrene in creosote-contaminated, clayey soil in systems with electric current and added microorganisms. They observed ten-fold higher degradation rates of phenanthrene in systems with current and microorganisms than in systems with neither. Viñas et al. (2005) studied biodegradation of PAHs of a creosote-contaminated soil and detected a reduction of PAHs by 83 - 87% during a 200-day experiment. They concluded that aeration and water content were the most important factors affecting biodegradation of PAHs. The results of these studies are very similar to the ones obtained in the studies described in Paper II. According to these studies, biodegradation combined with electrokinetics is a good option for treating creosote-contaminated soils. Inoculating the soil with indigenous microorganisms enriched with creosote did not appear to be necessary; a mixture of strains capable of degrading the target contaminants was already present in the test soil. Table 7 presents comparisons of the effects of biostimulation and bioaugmentation on pyrene and phenanthrene concentrations. The results are contradictory and no general conclusions can be drawn. The soil characteristics, contaminants and their concentrations, microbial population, and environmental conditions vary with each case. It is, therefore, difficult to predict the effect bioaugmentation might have on degradation efficiency.

**Table 7.** Comparison of biostimulation and biodegradation tests of phenanthrene and pyrene. Bioaugmentation tests used enrichment cultures from PAH-contaminated soils.

Contaminant initial concentration (mg/kg)	Bioaugmentation reduction-%	Biostimulation reduction-%	Reference
Phenanthrene (100) Pyrene (100)	96% 78%	48% 12%	Hwang and Cutright, 2002
Phenanthrene (86.8) Pyrene (99.3)	98% 19%	98% 98%	Yu <i>et al.</i> , 2005
Phenanthrene (111-115) Pyrene (183)	50% 45%	28% 22%	Negri <i>et al.</i> , 2004
Phenanthrene (496) Pyrene (387)	96% 89%	96% 90%	Viñas <i>et al.</i> , 2005

In some cases, inoculation may be beneficial. A mixture of strains is usually a better alternative as an inoculum than just one. Oil products and creosote contain hundreds of compounds and it is unlikely that one strain could actively degrade even most of them. It is also beneficial to use microorganisms enriched from the target soil with the target contaminant. The inoculated strains are already adapted to the conditions prevailing at the site and have a better chance of survival (El Fantroussi and Agathos, 2005). Inoculated organisms often do have difficulties to thrive on sites because of competition over nutrients and habitats and because they have not adapted to prevailing conditions. Nevertheless, even if they fail to survive, they may still enhance contaminant degradation. By transferring mobile plasmids containing genes for the degradation of contaminants from strain to strain on the site (horizontal transfer), they pass on the ability to degrade contaminants even if the inoculated strains themselves do not survive for long (Romantschuk *et al.*, 2000). Consequently, the degradation capacity of the indigenous microflora increases resulting in more active biodegradation.

Cost-efficiency is vital for any practical remediation technique. Several factors influence the cost of an *in situ* -electrokinetic process: (i) soil characteristics, (ii) moisture content, (iii) contaminants and their concentrations, (iv) concentrations of non-target ions and conductivity of the pore water, (v) depth of the remediated soil, (vi) site preparation requirements, and (vii) electricity and labour costs (van Cauwenberghe, 1997; Virkutyte *et al.*, 2002). Most of the studies of electrokinetic processes are laboratory-scale tests that usually omit cost estimates. However, Alshawabkeh *et al.* (1999) presented an example of a cost estimate for a contaminated site of saturated silty clay with a surface area of 5 000 m<sup>2</sup> and depth of 5 m. The estimated total cost of remediation was 40-48 \$US/m<sup>3</sup> (31-38 €/m<sup>3</sup>). Comparison of different treatment methods is very difficult because the parameters affecting costs vary at each site, even within a site. Virkutyte *et al.* (2002) did compare the cost of the Lasagna-project with the cost of soil heating/vapour extraction, and of chemical

oxidation: 50-120 \$US/m<sup>3</sup>, 85-160 \$US/m<sup>3</sup>, and 130-200 \$US/m<sup>3</sup>, respectively. When defining the cost-efficiency of electroremediation, the price of electricity naturally plays a key role. Even though the price of electricity has increased in the last few years, in Finland, electricity is not expensive by European standards. In Finland, the price of industrial electricity is 6.99 snt/kWh including taxes, in Germany or Netherlands 10.5-10.7 snt/kWh and in Italy 12 snt/kWh ([www.energia.fi](http://www.energia.fi)).

## 5.2. Oil sorbents

When designing a fabric to be used as a biodegradable first aid for removal of oil spills, several factors are important: The oil absorption capacity of the fabric has to be excellent; it has to have good physical characteristics, such as strength even when saturated with oil and resilience while in storage; and the fabric itself should be readily degradable after use and provide a good support for growth of the degrading microorganisms (Choi and Cloud, 1992; Deschamps et al., 2003; Teas et al., 2001). Natural sorbents are an attractive option due to their biodegradability (Choi and Cloud, 1992; Johnson et al., 1973; Setti et al., 1999). However, some natural sorbents are hydrophilic, which is a disadvantage for oil absorption in aqueous systems. The hydrophilic nature of some natural sorbents is probably due to treatments that have removed the natural waxes coating the surface of raw fibres. These waxes enhance the hydrophobic and oleophilic properties of natural sorbents (Johnson et al., 1973).

The study presented in Paper III showed that cotton grass fibre is an excellent sorbent for the removal of several oil types from the surface of water. Cotton grass fibres absorbed considerably more oil than the commercial, polypropylene sorbent (up to 27 g/g and ~10g/g, respectively). Low temperature and brackish water conditions did not reduce the diesel-absorption capacity of cotton grass; on the contrary, the highest absorption capacity was in icy conditions in artificial seawater. Choi and Cloud (1992) and Johnson et al. (1973) also noted an increase in the absorption capacity of milkweed and cotton fibres at low temperatures. The increased absorption is probably due to the increased viscosity of oil resulting in enhanced adherence of oil to the fibres (Choi and Cloud, 1992). This is a very important characteristic for a sorbent to be used in cold climate areas such as the Baltic Sea, where the average temperature is around 10 °C (HELCOM, 2003). Several natural fibres have proved to be efficient sorbents from the surface of water, such as cotton (absorption capacity of light crude oil ~35 g/g, Choi and Cloud, 1992), acetylated rice straw (machine oil up to 24 g/g, Sun et al., 2002), and milkweed (light crude oil ~ 40 g/g, Choi and Cloud, 1992). However, these plants do not grow at these latitudes. Cotton grass fibre is an excellent and cost-efficient choice for cold-climate areas, such as Finland.

Since cotton grass fibre is a by-product of peat excavation, the cost of raw material would be minimal. Additional costs will come from processing the fibres to a suitable form. The amount of purification of the fibre adds to the cost of the material, but purified fibre has better overall absorption properties than the plain, unpurified one. Existing bast



fibre processing machinery could possibly be useful in purifying processes. Määttä (2003) evaluated the cost of cotton grass in fibre form to cost around 200 €/m<sup>3</sup>, when the cost of sorbent peat and other sorbent materials were 100 €/m<sup>3</sup> and 500 €/m<sup>3</sup>, respectively. Peat is a cheaper material but its oil absorption capacity is lower: 3-4 g/g for diesel oil (Biswas et al., 2005; Ribeiro et al., 2000). Hence, cotton grass fibre is a very cost-efficient material for oil sorption. Another concern is the ease of handling. Purified fibre pressed into a mat makes handling of the material easier and reduces dust formation – but, again, will raise the cost of the product. The use of binding materials may improve the strength and ease of handling of the product, but non-biodegradable binding materials diminish its ecological value. Heat processing the cotton grass fibres seems to be unadvisable, as it appears to diminish the absorption capacity. Even after heat processing, the performance was still clearly better than that of the synthetic sorbent.

The degradation of oil in the oily sorbents may not be very rapid in certain conditions, even with a biodegradable fabric. Therefore, it was investigated whether addition of a suspension of microbes, enriched for the purpose, would improve the oil degradation rate once the cloth has been disposed of in compost or in soil. Good absorption of water might be considered as a disadvantage for an oil spill treatment fabrics, but for the purpose presented here, high hydrophobicity would result also in resistance against microbial colonisation and degradation. Because the oil itself is hydrophobic and thus poorly bioavailable, one role for the fabric is to disperse the oil into thinner layers enhancing its surface area. Greater surface area renders oil more accessible to microbes. For the same purpose, the ability to absorb water is also likely to improve the performance of the fabric. This aspect was not, however, addressed in the present study.

For the production of the microbially supplemented oil sorbent, a peat fabric was chosen. Peat absorbs water, which is essential for the activity of the microorganisms. When producing the microbially supplemented fabric, the microorganisms went through several growth-drying cycles to enrich those that tolerate drying well. Indeed, the viable counts in the cloths remained quite high and constant throughout the test period. Drying has been long used as a preservation method of microorganisms (Morgan et al., 2006). Although degradation of oil from the sorbents would be more efficient for example in a bioreactor, drying microbial suspension to the fabric was chosen as the means to add microbes, because the aim was to develop a material that would be easy to dispose of and that would not require active treatment techniques. The microbial treatment decreased the water/oil holding and absorption capacities, but the performance of the fabric was still quite good. (2.6 g/g and 3.2 g/g of motor oil, respectively). The microbial treatment appeared to enhance the amount of oil leaking from the cloths to the surrounding soil, resulting perhaps from the dissolving effect the microorganisms have on the oil. The dissolved oil was degraded in the vicinity of the cloths, however, so it would not pose an additional threat to the environment. The dried microbial suspension in the cloths did not appear to have a clear overall effect on the biodegradation of oil. In the first few weeks, the degradation was faster with microorganisms in the incubations in sand, but after 12 weeks, no significant difference in the loss of oil in the two cloth types was observable. Especially in garden soil, the indigenous microorganisms appeared to be capable of

degrading oil rapidly; therefore, the added microorganisms had no real stimulatory effect. Although the benefits of developing an effective biodegradable sorbent are clear, the benefits of attaching microorganisms to the sorbent may be insufficient.

Very few studies on biodegradation of oily sorbents exist. Setti et al. (1999) investigated biodegradation of heavy oil of a *Pseudomonas*-strain with the presence of an oil sorbent. The system mimicked inoculation of oil sorbents on the surface of water. In the presence of natural sorbents, keratin, chitosin, and chitin, biodegradation of n-alkanes was complete in 7 days and in their absence, in 40 days. Biswas et al. (2005) determined the effect of sorbents on the microbial uptake of diesel of a mixed culture of diesel degraders in batch slurry microcosms. The biodegradation rates were lower for two oil sorbents than for the control soil. However, the initial concentration of diesel in the sorbents was significantly higher than in the soil: 288-649 and 178 mg/kg, respectively. More studies are required to explore the biodegradation potential of oil from oily sorbents.

## 6. Conclusions and future prospects

This study proved that electrokinetics is beneficial for bioaugmentation because it facilitates dissemination of bacteria in soils with low permeability. When dealing with older contaminations, bioaugmentation is often unnecessary, however; biostimulation is usually sufficient for fast degradation. Bioremediation combined with electrokinetics remediated creosote-contaminated soil efficiently. Electrokinetically enhanced bioremediation offers clear advantage in the bacterial dissemination process, as well as in introduction of nutrients, electron acceptors, and heat. Electrokinetic heating of soil increases the activity of microorganisms resulting in effective degradation even at low ambient temperature – an important factor in cold climate areas.

Cotton grass fibre proved to be an excellent oil sorbent for collecting oil from the surface of water. Cotton grass performed exceptionally well compared with the commercial product even in brackish water and at low temperature – conditions prevailing at the Baltic Sea. Drying microbial suspension on biodegradable oil sorbents to enhance oil degradation did not appear to be necessary. Indigenous hydrocarbon degraders degraded oil rapidly from oily sorbents, especially in garden soil.

More full-scale field studies are necessary to assess the challenges of *in situ* - bioremediation processes, as are innovative new techniques to overcome their limitations. Although overall knowledge of the degradation processes themselves and of the microorganisms performing them is quite advanced, there is not enough information about how the results gained from the laboratory relate to remediation processes in the field. This requires extensive research of parameters connected to the bioremediation processes and their scale-dependences in laboratory-, pilot-, and field-scale. Even if the full field-scale trials may be expensive and complex, they are essential in filling the gaps in our knowledge how parameters change in full-scale systems.

Very few studies have focused on biodegrading oil from oily sorbents or on other treatment methods of the sorbents. Practical, efficient, ecological, and economical methods for treating used sorbents are required that fully take advantage of the biodegradable nature of natural sorbents. One aspect of developing cotton grass fibres to a commercial oil sorbent product is the ease of handling. Untreated cotton grass fibres tend to create dust and the collection of the fibres from the surface of water would be easier if the fibres were in a more practical format. The material could be produced into a mat or cloth, but the challenge is to find a suitable binding material: Many are available, but they are mostly synthetic, which would compromise the biodegradability.

## 7. Tiivistelmä

Öljyä käytetään maailmassa vuosittain valtavia määriä, minkä takia öljyvuodot maalla ja merellä ovat yleisiä. Öljytuotteiden lisäksi myös muut teollisesti käytetyt hiilivedyt, kuten kreosootti, pilaavat maaperää. Useimmat hiilivedyt ovat biologisesti hajoavia. Bioremediaatio, puhdistus mikrobien avulla, onkin houkutteleva vaihtoehto hiilivedyillä saastuneiden alueiden puhdistukseen. Kylmän ilmaston alueilla biohajotus on kuitenkin yleensä hidasta. Tämän väitöskirjan tarkoituksena on kehittää tehokkaita, edullisia ja ekologisia menetelmiä hiilivetyjen poistamiseen sekä veden ja kiinteiden aineiden pinnoilta että maaperästä kylmän ilmaston alueella.

Työssä tutkittiin hiilivedyillä saastuneen maan puhdistuksen tehostamista sähkökineettisesti. Tarkoituksena oli levittää ravinteita ja mikrobeja maaperään sähkön avulla ja samalla lämmittää maata, jotta saasteiden biosaatavuus ja mikrobien aktiivisuus lisääntyisivät. Sähköosmoosi osoittautui tehokkaaksi menetelmäksi levittää bakteereja ja ravinteita keski- tai hienorakeiseen maahan. Kreosootti, puunkyllästysaineena käytetty hiilivetyseos, hajosi nopeammin sähköosmoottisesti lisättyjen ravinteiden avulla kuin kontrolleissa ilman lisäyksiä. Rikastettujen, kreosoottia hajottavien mikrobien lisäys ei kuitenkaan ollut tarpeellista, sillä käsiteltävän maan omat mikrobit olivat sopeutuneet saasteeseen ja kykenivät sen tehokkaaseen hajotukseen.

Pienten öljyvuotojen puhdistamiseen käytetään yleensä öljynimeytysaineita. Useimmat nykyään käytössä olevat öljynimeytysmateriaalit ovat synteettisiä, jolloin poltto on ainoa vaihtoehto niiden loppukäsittelyyn. Biohajoavan imeytysmateriaalin voisi sen sijaan käsitellä esimerkiksi kompostointia muistuttavalla tavalla. Tupasvilla on turvesoiden yleinen kasvi, jonka vettähylykivä kuitu on turpeennoston sivutuote. Tupasvilla osoittautui erinomaiseksi öljynimeytysmateriaaliksi etenkin veden pinnalla olevan öljyn poistoon. Se imi jopa kolminkertaisesti öljyä synteettiseen, kaupallisesti saatavilla olevaan öljynimeytysaineeseen verrattuna. Tupasvilla toimi erittäin hyvin myös Itämeren talvea muistuttavissa olosuhteissa. Lisäksi tutkittiin öljyisten liinojen loppukäsittelyn tehostamista öljynimeytysliinaan kuivattujen, öljyä hajottavien mikrobien avulla. Mikrobilisäys saattaa olla hyödyllinen, jos loppukäsittely tapahtuu mineraalimaassa, jossa mikrobiitiheys on alhainen ja öljyn nopea hajotus on toivottavaa. Muutoin se tuskin on kannattavaa.

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